Uses of Banding Techniques for the Identification of Human Diseases of Cytogenetic Origin

by Margery W. Shaw*

The longitudinal differentiation of chromosomal segments by the development of special straining techniques has been one of the most exciting advances in human cytogenetics during the last four years. Prior to the quinacrine and modified Giemsa staining methods only partial differentiation of chromosome arms had been achieved. For example, in conventionally stained preparations it was possible to detect, in favorable cells, the secondary constrictions in the long arms of chromosome Nos. 1, 9, and 16. Also, polymorphism of the stalks and satellite regions of the short arms of the acrocentrics (13, 14, 15, 21, and 22) had been described, and family studies revealed that the variants were usually inherited. Autoradiography, using tritiated thymidine incorporation in the late S phase of DNA synthesis, allowed detection of segments of chromosomes which had completed synthesis later than other segments. But since there was a great deal of apparent asynchrony between homologs, the discovery of minor variants along the arms was hampered. However, the method did allow distinction between morphologically similar pairs (4 vs. 5; 13 vs. 14 vs. 15; 17 vs. 18).

The banding methods developed recently have been described in detail and will not be repeated here (*1*). A description of the mechanisms of chromosome banding and the relationship of bands to chromosome structure have been thoroughly discussed elsewhere (*2,3*).

There are four general banding patterns, called C-bands, Q-bands, G-bands, and R-bands. Table 1 gives the general techniques used for banding, the patterns which result, and selected references. Table 2 summarizes the comparison of the four banding patterns in certain regions of the chromosomes where inconsistent patterns have emerged.

The great promise of the banding techniques was the detection of minute rearrangements within the arms of the chromosomes (inversions, deletions, and duplications) as well as equal exchanges between chromosomes which did not perceptibly alter the arm lengths or arm ratios (reciprocal translocations). Evidence of this type has occurred but the accumulation has been slow. It had been hoped that many individuals with phenotypes or syndromes which suggested that a chromosomal abnormality might be present but in whom only "normal" chromosomes were found by conventional techniques would harbor a small rearrangement which could be demonstrated by finer analysis using banding methods. This hope has not yet been realized except in a few exceptional instances.

*University of Texas Graduate School of Biomedical Sciences at Houston, Texas 77025.

Table 1. Banding methods of human chromosomes.

Type	Band pattern	Methods	References
C-bands	Constitutive heterochromatin consisting of four regions: the centromere, the short arms of the acrocentrics, the secondary constrictions of 1, 9, and 16, and the distal long arm of the Y	Denaturation of DNA with sodium hydroxide; renaturation in 2X SSC; Giemsa stain	(*4,5*)
Q-bands	All arms of all chromosomes show specific bands ranging from little or no fluorescence through pale, medium, and intense, to brilliant fluorescence	Staining with quinacrine hydrochloride or quinacrine mustard followed by observation by use of ultraviolet microscopy.	(*6,7*)
G-bands	Except for the constitutive heterochromatin regions of Nos. 1 and 16 and the Y, the bands are precisely comparable to the Q-band patterns (i.e., intense fluorescence = dark Giemsa strain)	Many different techniques, including modification of the C-band methods, alkaline pH of Giemsa, trypsin and pronase treatment.	(*8–13*)
R-bands	Patterns produced are the reverse of G-bands except for the heterochromatin region of No. 9	G-band method, but increasing the temperature from 65 to 88°C.	(*14*)

Table 2. Comparison of staining and fluorescent intensity of the secondary constrictions and the Y heterochromatin.

	1qh	9qh	16qh	Distal Yq
C-band	+	+	+	+
Q-band	–	–	–	+
G-band	+	–	+	Variable
R-band	–	–	–	Variable

It is the purpose of this paper to describe the present state of our knowledge of chromosomal mutations discovered by banding techniques. But before discussing the question posed by the title of the paper—identification of human diseases of cytogenetic origin—we should first look at normal variation in banding patterns of human chromosomes which are not correlated with disease or defect.

Soon after Q-bands were observed, it became obvious that there was individual variation between homologs for certain segments of certain chromosomes (*1*). In some of these, the variation has been shown to follow Mendelian patterns of segregation in family studies. These regions are: 1qh, 3q, 4c, 9qh, 13p, 13q, 14p, 15p, 16qh, 21p, 22p and Yq (c = centromere; p = short arm; q = long arm; h = heterochromatic secondary constriction region).

Later, an unexpectedly high frequency of variation in C-bands was discovered (*15*). Among 20 phenotypically normal individuals, 31 C-band variants were detected comprising an increase, a decrease, or a misplacement of C-bands. These involved 1qh, 3q, 9qh, Dc, Ds, 16qh, Ec, Fc, Gc, and Gs (s = satellite; D = 13–15; E = 17–18, F = 19–20, G = 21–22). In two families, three out of six C-band variants were transmitted to an offspring. Further extension of these observations show 28 of 33 variants to be inherited from one parent while five arose *de novo*. Since mouse and human studies have indicated a precise correspondence between highly repetitious DNA and sites of C-banding (*16–20*), it is not unreasonable to predict that there may be variation in length of C-bands between generations in tandemly repeated sequences where unequal crossing over could occur.

So far, G-band and R-band variants have not been found in normal individuals, suggesting little or no chromosomal polymorphism in the vast majority of chromosomal segments.

One of the first applications of banding techniques of clinical significance was the confirmation that the cri-du-chat chromosome was No. 5 (*21*). This chromosome

had been previously identified as No. 5 by autoradiography and measurements (*22*).

An early unexpected finding from banding studies was the discovery that the Philadelphia (Ph^1) chromosome of chronic myelocytic leukemia is not the No. 21 chromosome, which is trisomic in Down's syndrome, as had been previously supposed. Instead, the Ph^1 chromosome is autosome No. 22 (*23, 24*). The earlier assumption had been based on the fact that leukocyte alkaline phosphatase levels were elevated in Down's syndrome and reduced in chronic myeloid leukemia.

Quite recently Rowley has discovered, in a series of 17 patients with chronic myelocytic leukemia who are Ph^1-positive, that a weakly fluorescent segment is added to the end of the long arm of chromosome No. 9 (*25*). This suggests that the Ph^1-lesion is a translocation rather than a chromosomal deletion.

It was predictable that banding studies would be used to reinvestigate patients with known structural rearrangements in an attempt to identify more precisely the chromosomal segments involved. Evans et al. (*26*) described a pericentric inversion of No. 3 in one individual and a heritable translocation between Nos. 9 and 22 in another. Francke (*27*) reported the first large series of reanalysis using the new cytogenetic techniques. She applied the fluorescence technique to 16 individuals in 11 families who had been studied previously by routine staining methods and autoradiography. In six families, the translocation chromosomes which had been classified by group were clearly identified by Q-bands. In two families, errors in identification were corrected, and in one case a new lesion was discovered. Her studies raised the possibility of nonreciprocal translocation, since in every instance there seemed to be one segment missing from one chromosome and added to another rather than two exchanges. Thus, the dogma of the integrity of the telomeres is questioned (*28*). On the other hand, there may be a nonrandom distribution of breaks with preference for the telomere regions.

Rings involving Nos. 18, 21, and 22 have been demonstrated by fluorescence (*29, 30*). A new autosomal trisomy syndrome (No. 8) has been established in at least five patients (*31, 32*), and also trisomy-8 is described in the bone marrow cells of patients suffering from various hematopoietic disorders (*33*). Rearrangements involving the sex chromosomes have been confirmed. An isochromosomes for the short arm of the X was verified by banding (*34*). A Y-autosome translocation was demonstrated by the presence of the intensely fluorescent Y body at the end of a translocation chromosome involving the long arm of 14 (*35*). A pericentric inversion of the Y was traced through four generations (*36*), and an unusually long X chromosome with an interstitial C-band has been described (*37*). There has also been a fruitless search for a fluorescent Y-body lurking in the karyotypes of XX males (*38*).

These are merely examples of the types of abnormalities in chromosomal disorders to which banding techniques have been applied. Many more have been reported but will not be discussed here. A more fertile field of investigation has been in the area of tumor chromosomes.

Banding patterns of long-term human lymphocyte lines and established cell lines have been investigated by Miller et al. (*39*). The WI–38 karyotypes were indistinguishable from those of normal females, the WI–L2 (lymphoblastoid) line was pseudodiploid, and three HeLa cell line derivatives as well as D98/AG all had several marker chromosomes in common suggesting they were all derived from HeLa.

A specific banding abnormality has been discovered in Burkitt's lymphoma (*40*). Among 12 individuals studied, 10 were shown to have an added Q-band (or G-band) at the end of the long arm of one No. 14. This occurred in both biopsy material and subsequent culture. It could not be determined whether the extra band originated as a translocation of a segment from another chromosome or as a duplication of a segment within No. 14.

In contrast to an extra band in Burkitt's lymphoma, there is at least one missing band

in some patients with retinoblastoma. Retinoblastoma is a dominantly inherited disorder which can be caused by either a germinal or somatic mutation. In the germinal form (usually familial and bilateral), a chromosomal lesion, if it exists, would be expected to be present in all tissues of the body. Wilson et al. (*41*) described one patient and summarizes the findings of three others in whom retinoblastoma and a 13q– or 13r chromosome coexisted. In their case, the interstitial deletion of the long arm involved the fourth band distal to the centromere. The findings in chronic myeloid leukemia, Burkitt's lymphoma, and retinoblastoma suggest that specific banding lesions are associated with tumors. Since there are many forms of inherited cancers (*42*) and many dominant disorders of late onset (*43*) these conditions may prove to be a fertile field for banding studies.

It is well established that malignant tissues harbor a wide range of chromosomal abnormalities, including aneuploidy, endoreduplication, polyploidy, multipolar spindles, chromosomal breaks, and rearrangements producing marker chromosomes. Chen and Shaw (*44*) have recently employed the Q-band, G-band, and C-band staining techniques in cells from a biopsy and subsequent culture of a human malignant melanoma. A modal chromosome number of 45 with seven marker chromosomes was found, including a ring and a large marker chromosome with an interstitial C-band. These rearrangements were present *in vivo*, since they were observed in metaphases 4 hr after biopsy and they persisted *in vitro* during 11 weeks of subsequent culture. Chen (personal communication) has now established six human tumor strains in culture—four from melanoma and one each from gastric adenocarcinoma and leiomyosarcoma. Two were hypodiploid (41–45), one hyperdiploid (48–52), two hypotriploid (55–65) and one hypotetraploid (79–83). Homogeneity and stability have been confirmed in four of the six newly established strains. He finds that the degree of chromosomal rearrangements is much farther advanced than originally expected. There is a minimum cumulative value of 13 chromosomal abnormalities in each of these cultures.

How can banding techniques be applied to mutagenesis studies? There are no obvious direct applications in the offing because of two limitations. First, banding analysis is laborious and time-consuming and thus not practical for large-scale screening studies involving many cells in many individuals. Second, small rearrangements, detected by banding differences, would be expected to be less frequent than the gross but easily detectable unstable chromosomal aberrations (breaks, acentric fragments, dicentrics, exchanges, etc.) which are scored by conventional methods of clastogen screening. This latter limitation is true because only a fraction of the aberrations produced would be expected to mimic otherwise normal-appearing chromosomes and survive several cell generations. Nevertheless, the technical difficulties of banding screening may be overcome by the application of automated densitometry methods and computer analysis of banded chromosomes.

Acknowledgements

Permission to use unpublished findings of Ms. Ann Craig-Holmes and Dr. T. R. Chen is gratefully acknowledged. Their work has been supported by USPHS grant 1 PO1 GM19513.

REFERENCES

1. Bergsma, D., Ed., Paris Conference, 1971: Standardization in Human Cytogenetics. The National Foundation Original Article Series, New York, 1973.
2. Comings, D. E. The structure and function of chromatin. In: Advances in Human Genetics, Vol. 3. H. Harris and K. Hirschhorn, Eds., Plenum Press, New York, 1972, pp. 237–431.
3. Comings, D. E., et al. The mechanism of C- and G-banding of chromosomes. Exp. Cell Res., 77: 469 (1973).
4. Pardue, M. L., and Gall, J. G. Chromosomal localization of mouse satellite DNA. Science 168: 1356 (1970).
5. Arrighi, F. E., and Hsu, T. C. Localization of heterochromatin in human chromosomes. Cytogenetics 10: 81 (1971).

6. Caspersson, T., et al. Chemical differentiation along metaphase chromosomes. Exp. Cell Res. 49: 219 (1968).
7. Caspersson, T., et al. Chemical differentiation with fluorescent alkylating agents in Vicia faba metaphase chromosomes. Exp. Cell. Res. 58: 128 (1969).
8. Sumner, A. T., Evans, H. J., Buckland, R. A. New technique for distinguishing between human chromosomes. Nature New Biol. 232: 31 (1971).
9. Drets, M. E., and Shaw, M. W. Specific banding patterns of human chromosomes. Proc. Nat. Acad. Sci. U.S. 68: 2073 (1971).
10. Patil, S. R., Merrick, S., and Lubs, H. A. Identification of each human chromosome with a modified Giemsa stain. Science 173: 831 (1971).
11. Schnedl, W. Analysis of the human karyotype using a reassociation technique. Chromosoma 34: 448 (1971).
12. Seabright, M. A rapid banding technique for human chromosomes. Lancet 2: 971 (1971).
13. Dutrillaux, B., et al. Mise en evidence de la structure fines des chromosomes humains par digestion enzymatique (pronase en particulier). C. R. Acad. Sci. Paris, Ser. D 273: 587 (1971).
14. Dutrillaux, B., and J. Lejeune. Sur une nouvelle technique d'analyse du caryotype humain. C. R. Acad. Sci. Paris, Ser. D 272: 2638 (1971).
15. Craig-Holmes, A. P., Moore, F. B., and Shaw, M. W. Polymorphism of human C-band heterochromatin: frequency and family studies. Amer. J. Hum. Genet. 25: (2): 181 (1973).
16. Jones, K. W. Chromosomal and nuclear location of mouse satellite DNA in individual cells. Nature 225: 912 (1970).
17. Jones, K. W., and Corneo, G. Location of satellite and homogeneous DNA sequences on human chromosomes. Nature New Biol. 233: 268 (1971).
18. Jones, K. W., and Robertson, F. W. Localization of reiterated nucleotide sequences in *Drosophila* and mouse by *in situ* hybridization of complementary RNA. Chromosoma 31: 331 (1971).
19. Pardue, M. L., and Gall, J. G. Chromosomal localization of mouse satellite DNA. Science 168: 1356 (1970).
20. Jones, K. W., and Corneo, G. Location of satellite and homogeneous DNA sequences on human chromosomes. Nature New Biol. 233: 268 (1971).
21. Caspersson, T., Lindsten, T. J., and Zech, L. Identification of the abnormal B group chromosome in the "cri du chat" syndrone by QM-fluorescence. Exp. Cell Res. 60: 315 (1970).
22. Warburton, D., et al. Distinction between chromosome 4 and chromosome 5 by replication pattern and length of long and short arms. Amer. J. Human Genet. 19: 339 (1967).
23. Caspersson, T., et al. Identification of the Philadelphia chromosome as No. 22 by quinacrine mustard fluorescence analysis. Exp. Cell Res. 63: 238 (1970).
24. O'Riordan, M. L., et al. Distinguishing between the chromosomes involved in Down's syndrome (Trisomy 21) and chronic myeloid leukemia (Ph^1) by fluorescence. Nature 230: 167 (1971).
25. Rowley, J. D. A new consistent chromosomal abnormality in chronic myelogenous leukemia. Nature 243: 290 (1973).
26. Evans, H. J., Buckton, K. E., and Sumner, A. T. Cytological mapping of human chromosomes: Results obtained with quinacrine fluorescence and the acetic-saline-Giemsa techniques. Chromosoma 35: 310 (1971).
27. Francke, U. Quinacrine mustard fluorescence of human chromosomes: Characterization of unusual translocations. Amer. J. Human Genet. 24: 189 (1972).
28. Shaw, M. W. Human chromosome abnormalities revisited. Amer. J. Human Genet. 24: 227 (1972).
29. Cohen, M. M., Storm, D. F., and Capraro, V. J. A ring chromosome (No. 18) in a cyclops. Clin. Genet. 3: 249 (1972).
30. Crandall, B. F., et al. Identification of 21r and 22r chromosomes by quinacrine fluorescence. Clin. Genet. 3: 264 (1972).
31. de Grouchy, J., Turleau, C., and Leonard, C. Etude en fluorescence d'une trisomie C mosaique probablement 8: 46, XY/47, XY, ?8+. Ann. Génétique 14: 69 (1971).
32. Caspersson, T., et al. Four patients with trisomy 8 indentified by the fluorescence and Giemsa techniques. J. Med. Genet. 9: 1 (1972).
33. de la Chapelle, A., Schröder, J., and Vuopio, P. 8-Trisomy in the bone marrow. Report of 2 cases. Clin. Genet. 3: 470 (1972).
34. de la Chapelle, A., Schröder, J., and Pernu, M. Isochromosome for the short arm of X, a human 46, XXpi syndrome. Ann. Hum. Genet. 36: 79 (1972).
35. Krmpotic, E., et al. Localization of male determining factor on short arm of Y chromosome. Clin. Genet. 3: 381 (1972).
36. Grace, H. J., Ally, F. E., and Paruk, M. A. 46, Xinv (Yp^+q^-) in four generations of an Indian family. J. Med. Genet. 9: 293 (1973).
37. Disteche, C., et al. An abnormal large human chromosome identified as an end-to-end fusion of two X's by combined results of the new banding techniques and microdensitometry. Clin. Genet. 3: 388 (1972).
38. George, K. P., and Polani, P. E. Y heterochromatin and XX males. Nature 228: 1215 (1970).
39. Miller, O. J., et al. Quinacrine fluorescent karyotypes of human diploid and heteroploid cell lines. Cytogenetics 10: 338 (1971).
40. Manalov, G., and Manalova, Y. Marker band in

one chromosome 14 from Burkitt's lymphoma. Nature 237: 33 (1972).
41. Wilson, M. G., Towner, J. W., and Fujimoto, A. Retinoblastoma and D-chromosome deletions. Amer. J. Human Genet. 25: 57 (1973).
42. Knudson, A. G., Strong, L. C., and Anderson, D.E. *Heredity and cancer in man. In: Progress* in Medical Genetics, Vol. IX, Grune and Stratton, New York, 1973, pp. 113–158.
43. MuKusick, V. A. Mendelian Inheritance in Man, 3rd ed., Johns Hopkins Press, Baltimore, 1971.
44. Chen, T. R., and Shaw, M. W. Stable chromosome changes in a human malignant melanoma *Cancer Res.* 33: 2042 (1973).